

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

PCT

To:

see form PCT/ISA/220

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY (PCT Rule 43bis.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.
PCT/JP2004/009370

International filing date (day/month/year)
25.06.2004

Priority date (day/month/year)
27.06.2003

International Patent Classification (IPC) or both national classification and IPC
C07K14/505, A61K48/00, C12N5/06, C12N15/62

Applicant
DNAVEC RESEARCH INC.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☒ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☒ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. FURTHER ACTION

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1bis(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

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Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.
 - ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).
2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:
 - a. type of material:
 - ☒ a sequence listing
 - ☐ table(s) related to the sequence listing
 - b. format of material:
 - ☒ in written format
 - ☒ in computer readable form
 - c. time of filing/furnishing:
 - ☐ contained in the international application as filed.
 - ☐ filed together with the international application in computer readable form.
 - ☒ furnished subsequently to this Authority for the purposes of search.
3. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
4. Additional comments:

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Box No. II Priority

1. ☐ The following document has not been furnished:

☐ copy of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(a)).

☐ translation of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(b)).

Consequently it has not been possible to consider the validity of the priority claim. This opinion has nevertheless been established on the assumption that the relevant date is the claimed priority date.

2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43*bis*.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. ☒ It has not been possible to consider the validity of the priority claim because a copy of the priority document was not available to the ISA at the time that the search was conducted (Rule 17.1). This opinion has nevertheless been established on the assumption that the relevant date is the claimed priority date.

4. Additional observations, if necessary:

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

- ☐ the entire international application,
- ☒ claims Nos. 1-11

because:

- ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):
- ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):
- ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.
- ☒ no international search report has been established for the whole application or for said claims Nos. 1-11
- ☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:
 - the written form ☐ has not been furnished
 - ☐ does not comply with the standard
 - the computer readable form ☐ has not been furnished
 - ☐ does not comply with the standard
- ☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-bis of the Administrative Instructions.
- ☐ See separate sheet for further details

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Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes: Claims	
	No: Claims	1-6,8-15
Inventive step (IS)	Yes: Claims	7
	No: Claims	
Industrial applicability (IA)	Yes: Claims	12-15
	No: Claims	

2. Citations and explanations

see separate sheet

Box No. VI Certain documents cited

1. Certain published documents (Rules 43bis.1 and 70.10)

and /or

2. Non-written disclosures (Rules 43bis.1 and 70.9)

see form 210

Ad Section I: Basis of the opinion

1. Reference is made to the following documents:

- D1: KUME AKIHIRO ET AL: "In vivo expansion of transduced murine hematopoietic cells with a selective amplifier gene." THE JOURNAL OF GENE MEDICINE. MAR 2003, vol. 5, no. 3, March 2003 (2003-03), pages 175-181, XP009039186 ISSN: 1099-498X
- D2: HANAZONO Y ET AL: "In vivo selective expansion of gene-modified hematopoietic cells in a nonhuman primate model" GENE THERAPY, vol. 9, no. 16, August 2002 (2002-08), pages 1055-1064, XP002303770 ISSN: 0969-7128
- D3: NAGASHIMA TAKEYUKI ET AL: "New selective amplifier genes containing c-Mpl for hematopoietic cell expansion." BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 303, no. 1, 28 March 2003 (2003-03-28), pages 170-176, XP002303771 ISSN: 0006-291X
- D4: JIN LIQING ET AL: "In vivo selection using a cell-growth switch" NATURE GENETICS, vol. 26, no. 1, September 2000 (2000-09), pages 64-66, XP002303772 ISSN: 1061-4036
- D5: KROSL JANA ET AL: "Interleukin-3 (IL-3) inhibits erythropoietin-induced differentiation in Ba/F3 cells via the IL-3 receptor alpha subunit" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 271, no. 44, 1996, pages 27432-27437, XP002303773 ISSN: 0021-9258
- D6: SHIKAMA YAYOI ET AL: "A constitutively activated chimeric cytokine receptor confers factor-independent growth in hematopoietic cell lines" BLOOD, vol. 88, no. 2, 1996, pages 455-464, XP002303774 ISSN: 0006-4971

Ad Section II :Priority

- 2) The priority document pertaining to the present application was not available at the time of establishing this written opinion. Hence, it is based on the assumption that all claims enjoy priority rights from the filing date of the priority document. If it later turns out that this is not correct, the P document cited in the international search report could become relevant to assess whether all claims satisfy the criteria set forth in Article 33(1) PCT.

Ad Section III :Non-establishment of opinion

3. Claims 1-11 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

Ad Section V :Reasoned statement under Rule 66.2(a)(ii); citations and explanations supporting such statement

4. Novelty (Article 33 (2) PCT)

- 4.1 D1 Kume et al. describes 'selective amplifier genes' (SAGs) that encode chimeric proteins that are a fusion of granulocyte colony-stimulating factor receptor and the steroid-binding domain. Prototype SAGs conferred estrogen-responsive growth on murine hematopoietic progenitors. A detailed study of lineage showed a preferential expansion of EGFP(+) cells in granulocytes and monocytes following 4-hydroxytamoxifen administration. A granulocyte colony-stimulating factor receptor was linked to the estrogen receptor (see abstract). Bone marrow cells were transduced with the retroviral construct (see p.177 col.1 third paragraph). Subsequently SAG-transduced cells were tracked in a murine bone marrow transplantation model. Analysis of the impact of 4-hydroxytamoxifen stimulation was investigated (see p.178 col.1 2 full paragraph).
- 4.2 D2 Hanazono et al. describes a selective amplifier gene (SAG) consisting of a chimeric gene composed of the granulocyte colony-stimulating factor (G-CSF) receptor gene and the oestrogen receptor gene hormone-binding domain (see Fig.1). In the present study, the efficacy of the SAG in the setting of a clinically applicable cynomolgus monkey transplantation protocol was evaluated. Cynomolgus bone marrow CD34+ cells were transduced with retroviral vectors encoding the SAG and reinfused into each myeloablated monkey. Even with nonmyeloablative conditioning, successful engraftment of transduced cells even at low levels may allow expansion to clinically relevant levels with this method (see p.1059 col.1 1 full §). A modified SAG with thrombopoietin receptor (Mpl) as a growth signal generator instead of G-CSF receptor to overcome variable responses among monkeys is proposed (see p.1060 col.2 last sentence of the 1

full paragraph).

- 4.3 D3 Nagashima et al. describes the in vitro cell expansion with modified SAGs containing the thrombopoietin (TPO) receptor (c-Mpl) gene instead of GCR as a more potent signal generator.
- 4.4 D4 Jin et al. describes the successful in vivo expansion of gene modified haematopoietic cells using the cell growth switch composed of the intracellular part of Mpl and FKBP in a murine model. FKBP is a cytokine receptor-FK506 binding protein.

Thus, in view of the content of D1, D2, D3, D4 claims 1-6,8-13 lack novelty.

- 4.5 D5 Krosi et al. discloses that a chimeric receptor of the extracellular domain of the EpoR and the transmembrane and intracellular domains of IL-3R-beta-_{IL-3} chain (EpoR/IL-3R-beta-_{IL-3}) was capable of Epo-induced proliferative and differentiating signalling. An EpoR/IL-3R-alpha chimera, in contrast, was capable of transmitting a weak Epo-induced proliferative signal but failed to stimulate accumulation of beta-globin mRNA (see abstract). EpoR chimeric cDNAs were generated (see materials and methods).
- D6 Shikama et al. constructed four hybrid receptors: the extracellular region of either murine nEpoR or cEpoR linked to the transmembrane and cytoplasmic regions of either the human GMR-alpha or beta-c subunit (nE-alpha, nE-beta, cE-alpha, and cE-beta). Expression nEpo-beta led to Epo-dependent growth (see abstract). Hybrid and full length receptor were constructed and transfected into BaF3 or CTLL-2 cell lines (see materials and methods).

In view of the content of D5 and D6, claims 14 and 15 lack novelty.

5) Amendments (Article 19(2)/Article 34(2)(b) PCT)

The attention of the applicant is drawn to the fact that the application may not be amended in such a way that it contains subject-matter which extends beyond the content of the application as filed, Article 19(2)/Article 34(2)(b) PCT.

In order to facilitate the examination of the conformity of the amended application with the requirements of Article 34(2)(b) PCT, the applicant is requested to clearly

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identify the amendments carried out, no matter whether they concern amendments by addition, replacement or deletion, and to indicate the passages of the application as filed on which these amendments are based (see also Rule 66.8(a) PCT).

If the applicant regards it as appropriate these indications could be submitted in handwritten form on a copy of the relevant parts of the application as filed.